



gene. Commercially, human interferon- $\alpha$  is manufactured by stimulating the Namalwa human lymphoblastoid cell line with Sendai virus to produce a natural mixture of at least 21 subtypes of interferon- $\alpha$ , which are then purified by chromatography to a purity of 95% and a specific activity of about  $100 \times 10^6$  IU/mg protein. Such a product, identified as human interferon  $\alpha$ -N1, is commercially available. It is also possible to prepare human interferon- $\alpha$  using recombinant DNA technology.

Since the early 1980s, advances in production techniques led to the use of both natural and recombinant human interferon- $\alpha$  for the treatment of chronic HBV. Nowadays, interferon- $\alpha$  is generally accepted as the standard agent for treatment of chronic HBV infection. Although this treatment can be regarded as successful in many cases, response rates to treatment with human interferon- $\alpha$ , as judged by sustained loss of viral markers, are generally considered to be less than 50%. For an extensive review of the use of interferons in the treatment of hepatitis, reference is made to the book "Interferons in the Treatment of Chronic Virus Infection of the Liver" by Eddleston and Dixon, Pennine Press, 1990.

Nucleoside analogues form a new generation of drugs used in the treatment of hepatitis B virus. They have been found to show strong *in vitro* activity and low toxicity. There are three main nucleoside analogues, designated lamivudine, adefovir and entecavir. They all act on the reverse transcriptase enzyme of the hepatitis B virus, but on different priming sites. Lamivudine has been tested in phase III trials.

Adefovir, or [9-(2-phosphonylmethoxyethyl)adenine], or its orally available prodrug adefovir dipivoxil (the [bis(pivaloyloxymethyl)ester prodrug] is *inter alia* described in Antimicrob. Agents Chemother., 1998, 42(7), 1620-8, and Hepatology 1999, 29(6), 1863-9. The drug is currently under investigation in phase III trials for its anti-HIV activity.

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and HBsAg) can be achieved in patients infected with HBV, particularly chronic HBV, by a combined treatment, using both a nucleoside analogue and interferon- $\alpha$ , in a specific protocol of administration. The invention thus relates to a method of treating a human patient infected with hepatitis B virus, wherein during a period of at least 26 weeks a nucleoside analogue and interferon- $\alpha$  are both administered to said patient.

By administering both a nucleoside analogue and interferon- $\alpha$  in the specific protocol according to the present treatment, it has been found possible to achieve significantly better results than with the conventional treatments involving only one of a nucleoside analogue and interferon- $\alpha$ . Moreover, the present treatment has been found to be successful in a number of cases wherein conventional treatment involving only one of said agents did not provoke a response.

The type of nucleoside analogue may be chosen from the group of lamivudine, adefovir and entecavir. This includes of course prodrugs of all nucleoside analogues, such as adefovir dipivoxil. Particularly good responses have been found using lamivudine. The nucleoside analogue may be administered in the form of any pharmaceutical formulation which can conventionally be used. Examples of possible formulations of lamivudine can be found in WO-A-98/42321. Preferably, the nucleoside analogue is administered in the form of an oral dosage form. For an overview of different nucleoside analogues, reference is made to *inter alia* Clin. Pharmacokinet. 1999, 36(2), 127-43, Antimicrob. Agents Chemoter. 1998, 42(12), 3200-17.

The dose wherein lamivudine is administered is preferably chosen between 50 and 150 mg per day for the period wherein the combined treatment is carried out. Adefovir may be administered in a dose of between 5 and 30 mg per day, whereas entecavir can be administered in a dose of between 0.01 and 1 mg per day.

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The subtype of interferon- $\alpha$  used in accordance with the invention is not critical. It is envisaged that the interferon- $\alpha$  may be in any suitable form or formulation. In a preferred embodiment, pegylated interferon- $\alpha$  (PEG-IFN) is used. This form of interferon- $\alpha$ , which is known to the person skilled in the art, can be administered less often and has less adverse effects than standard interferon- $\alpha$ . PEG-IFN need only be administered once or twice a week, whereas of standard interferon- $\alpha$  three injections per week are necessary.

The dose wherein interferon- $\alpha$  is administered is preferably chosen between 30 megaUnits (equivalence for PEG-IFN 100  $\mu$ g) and 15 megaUnits (equivalence for PEG-IFN 50  $\mu$ g) per week for the period wherein the combined treatment is carried out. It is preferred that the dose of interferon- $\alpha$  is decreased during treatment, particularly in case of debilitating adverse effects. Preferably, the first part of the period wherein both agents are administered, a higher dose is used than in the second part. In this embodiment, in the first part of the period the dose is preferably 30 megaUnits per week and in the latter period preferably 15 megaUnits per week.

An important aspect of the invention is that during a period of at least 26 weeks both agents are administered to a patient. Preferably, this period is at least 30 weeks. During this period, the agents may be administered at intervals ranging from daily to weekly. It is preferred that the nucleoside analogue is administered daily, while interferon- $\alpha$  is preferably administered once a week. The period wherein both agents are administered will usually not be longer than 52 weeks, as it has been found that longer periods of treatment may be poorly tolerated.

In accordance with the invention it is possible that the period wherein the combined treatment is carried out is preceded or followed by a period wherein only one of the two agents is administered.

Figures 1 and 2 describe the frequency and substance of patient monitoring during a treatment according to the invention.

The invention will now be elucidated by the  
10 following, non-restrictive examples.

15 To enhance the efficacy of antiviral therapy to achieve viral clearance (HBeAg negativity) in chronic hepatitis B patients by giving 52 weeks combination therapy of lamivudine and interferon- $\alpha$  (IFN).

All patients will receive lamivudine 150 mg per day in combination with IFN 30 megaUnits (MU) per week. After 32 weeks IFN will be reduced to 15 MU per week. Therapy will be given for a total treatment period of 52 weeks.

Weeks		0	8	16	24	32	40	48	52	68	78	
IFN		IFN 30 MU/wk				IFN 15 MU/wk				Follow-up		
Lamivudine		Lamivudine 1 x dd 150 mg										

30            Potential candidates for treatment are all patients  
older than 18 years, with a chronic compensated HBV infection  
(HBsAg positive, HBV DNA positive by hybridization assay) and

ALT>2xULN. These patients will be given an information sheet/consent form.

#### SCREENING POTENTIAL CANDIDATES (-4 WEEKS)

5 Collect eligibility data: history, physical examination, lab hematology, chemistry and virology, pregnancy test.

Check inclusion criteria: informed consent, ALT>2xULN,  $\geq 18$  yrs, anticonception.

10 Check exclusion criteria: evidence of other viral, alcoholic drug, hereditary or auto-immune hepatitis, decompensated liver disease, other significant medical illness or condition, pregnancy or contra-indication for interferon therapy.

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#### BASELINE VISIT (-2 WEEKS)

Discuss decision about starting therapy.

#### MONITORING

20 In accordance with the schemes shown in Figures 1 and 2. Quantitative hepatitis B virus DNA assessment (HBV DNA) was performed by Hybrid Capture assay I (Digene, Murex, detector limit  $1.5 \times 10^6$  Eurohep genome equivalent per ml) and if negative, by quantitative PCR (PCRQ, Roche Amplicar, 25 detection limit  $10^3$  Eurohep genome equivalent per ml). (See also J. Viral Hepatitis, 1998, 5, 307-312 on limiting dilution polymerase chain reaction in chronic hepatitis B patients, and Hepatology 1999 30, 238-43 on HBeAg and HBsAg in accordance with interferon- $\alpha$  for chronic Hepatitis B 30 infection, the contents of which are incorporated herein by reference). Figure 3 shows the results (HBV DNA and HBeAg) for a 23 year old woman having a known HBV infection since 1 year.

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